REMARKS

In the Office Action of May 29, 2009, prior Examiner Guzo informed Applicants that claims covering the process of the invention for isolating nucleic acids would be allowable if the process was limited to employing non-siliceous membranes having pore sizes in the range of 1 μm to 50 μm. See, paragraph bridging pages 7 and 8, Office Action of May 29, 2009. Accordingly, Applicants filed the fourth Request for Continued Examination in this record with an amended set of claims reciting the limitation on membrane pore size. See, Applicants' Submission Under 37 CFR § 1.114(c), dated November 28, 2008. In the current Office Action, dated February 11, 2009, Examiner Ketter has withdrawn the prior notice to Applicants of allowable subject matter and has rejected the claims as obvious in view of newly cited US Patent No. 5,858,700 ("Ausich"). The Examiner stated:

> "Ausich et al. teaches a method of isolating a biological molecule on a filter, wherein it is suggested that the pore size be increased to avoid clogging, indicating that this was well-known in the art. Pore sizes of 8 to 400 microns is taught.
>
> "It would have been obvious to one of ordinary skill in the art to have practiced the method of Ogawa et al. using larger pores as suggested by Ausich et als., motivated by Ausich et al., to avoid clogging by so doing.
>
> "To the extent that Applicants' comments in the remarks filed 28 November 2008 might apply to the instant rejection, it is believed that the addition of the Ausich et al. reference addresses those comments." (page 3, Office Action, dated February 11, 2009).

Thus, according to the Examiner, Ausich provides the teaching in the prior art that was missing in previous combinations of references in this record to render Applicants' claims obvious.

The process of Ausich is specifically designed for producing and isolating relatively large **crystals** of the pigment lycopene:

> "Large crystal size is important to obtaining lycopene at the purity desired. To obtain the desired large crystals **(average size about 0.01 to about 0.5 mm)**, the concentrations of the four constituents of the saponification reaction mixture are preferably present at about 25 to about 50 weight percent lycopene-containing oleoresin, . . ." (col. 7, lines 41-46, Ausich; emphasis added).

Such lycopene crystals are produced according to Ausich from an organic solvent (e.g., hexane) extract of a biological source of lycopene (e.g., tomatoes) in which the extract ("oleoresin") is subjected to a

saponification reaction, which in turn is diluted with water to yield relatively large insoluble crystals that can then be collected by methods known in the art for collecting crystals:

> "The saponification reaction cleaves proteins present in the cellular debris as well as in the fatty acids from the di- and triglycerides and phosphonates, decreasing the solubility of the lycopene in the resulting medium **so that crystals form** . . .
>
> "The saponified reaction mixture so formed is then diluted by admixture with low ionic strength (deionized) water, preferably warm, e.g. about 40°-80°C . . .
>
> "If the temperature is too cold, the diluted reaction mixture can become too viscous to filter. If the temperature is too hot, the diluted reaction mixture can foam, which interferes with crystal recovery.
>
> . . .
>
> "The diluted reaction mixture formed by addition of water is gently agitated until homogeneous and then either pumped or otherwise fed into **a filtration device that collects the crystals**. Virtually any filtration device known in the art can be used. In a preferred embodiment of the invention, the mixture is fed into a centrifuge basket filter having a 8-400 μm maximum pore size. As is well known, the larger pore sizes have fewer problems with clogging of the filter." (See, in Ausich, col. 8, line 42 - col. 9, line 11).

Accordingly, depending on the size of crystals (0.01 mm (10 μm) to 0.5 mm (500 μm)) obtained, it is not surprising that a batch of lycopene pigment crystals generated by the process of Ausich could be collected on a membrane that has pores of appropriate size, such as standard WHATMAN® No. 4 filter paper (which are known to retain particles 20 μm to 25 μm) as in Example 1 of Ausich or a glass sintered funnel that has a pore size of 25 μm to 50 μm as in Example 3 of Ausich.

Applicants respectfully submit that the above excerpts show that Ausich provides no more than a recitation of a standard step of collecting relatively large pigment crystals on a filter owing to the fact that the crystals remain insoluble and sufficiently large so as not pass through the pores of the filter. As explained below, Applicants further submit that the Examiner's belief that a person of ordinary skilled in the art would apply the membranes used for pigment crystal collection in Ausich to the method of Ogawa for isolating nucleic acids is in fact contrary to the explicit teaching of Ogawa for using ultrafiltration to isolate phage DNA on an appropriately selected ultrafiltration membrane (ultrafilter). Moreover, even if Ausich were combined with Ogawa and the secondary references already of record, the combination would not work. In contrast, only Applicants' claimed process for isolating nucleic acids that comprises **binding** the nucleic acids to one side of a non-siliceous membrane in the presence of an immobilization buffer permits the use of membranes with pores as large as 1 μm to 50 μm.

Applicants have thoroughly explained in this record the standard technique of ultrafiltration for separating biomolecules, such as nucleic acids and proteins, from smaller molecules by size exclusion on selected membrane filters and the specific use of ultrafiltration according to Ogawa for isolating naked DNA from phage M13 particles. See, Applicants' Response, dated February 7, 2008, and Applicants' Response and accompanying Declaration of Uwe Olemüller Pursuant to 37 CFR 1.132, submitted May 18, 2007. Briefly, ultrafiltration is a method of separating biomolecules that typically employs membranes (also called ultrafilters) that have pore sizes in the range of 10 angstroms (0.001 μm) to 1000 angstroms (0.100 μm) to separate species ranging in molecular weight from 10,000 daltons to 1,000,000 daltons. Membranes having a pore size greater than 0.100 μm are in fact quite large with respect to the size of biomolecules such as DNA, RNA, and proteins. For example, membranes having pores sizes of 0.22 μm or 0.45 μm are typically employed in the art to filter out intact bacterial cells or cell fragments from a liquid sample. Accordingly, a person of ordinary skill in this art understands that membranes with pore sizes greater than 0.100 μm are clearly not useful for separating nucleic acids from other biomolecules on the basis of size exclusion.

In the Example of Ogawa, a medium (pre-filtered to remove cells) containing suspended M13 phage particles is applied to an ultrafiltration membrane (ultrafilter) that has a fractionation molecular weight of 300,000 daltons in order to remove low molecular weight components in the culture medium. The phage particles are retained on the ultrafilter and then decomposed with proteinase K to release the naked phage DNA. The proteinase K and decomposed phage capsule proteins are then washed through the pores of the ultrafilter, leaving the naked phage DNA, which is larger than the pore size of the ultrafilter, retained on the ultrafilter (see, col. 4, lines 23-39, of Ogawa). As noted in Applicants' Response and accompanying declaration by Dr. Oelmüller, submitted May 18, 2007, an ultrafilter having a fractionation molecular weight of 300,000 daltons should have a pore size in the range of 0.035 μm and thus would be expected to retain the desired larger phage DNA and allow smaller molecules, such as the proteinase K and the decomposed phage proteins, to be easily washed through and away from the retained naked phage DNA. Ogawa also mentions that a preferred ultrafiltration membrane useful in the process has a fractionation molecular weight of 20,000 daltons to 1,000,000 daltons and notes that if the fractionation molecular weight is smaller than this range, then the efficiency of the removal of the decomposed proteins is reduced, **while if the fractionation molecular weight of the ultrafilter is larger than this range, the phage DNA may pass through the ultrafilter** (see, col. 3, lines 31-38, of Ogawa).

The above comments and others by Applicants and Dr. Oelmüller that are already in this record regarding ultrafiltration and Ogawa clearly show that the Examiner's attempt to combine the use of membranes having pore sizes as high as 8 μm to 400 μm according to Ausich to the ultrafiltration method of Ogawa to isolate nucleic acids is simply contrary to the goal and teaching of Ogawa for isolating phage

DNA from contaminating proteins and smaller molecules. Accordingly, the person of ordinary skill in this art would not be motivated to combine Ausich with Ogawa and any of the other references cited in this record to obtain Applicants' claimed process.

As already explained in this record, **Applicants' claimed process does not employ ultrafiltration** wherein any molecule that is larger than the size of the pores of a selected membrane is physically prevented from passing through the membrane while molecules smaller than the pore size pass through the filter. In particular, Applicants' claimed process is based on the surprising discovery that nucleic acids can be isolated in a process in which the nucleic acids are immobilized on one side of a non-siliceous membrane **by binding the nucleic acids to the membrane in the presence of an immobilization buffer** wherein the membrane possesses pore sizes from 1 μm to 50 μm that would otherwise permit the unbound nucleic acids to pass through the membrane.

By way of example, as shown in Table 5 of Example 5 at page 25 of Applicants' specification, total RNA was isolated from HeLa cells using non-siliceous membranes having pore sizes ranging from 0.01 μm to 20 μm. Persons skilled in the art would appreciate that the range of pore sizes of the non-siliceous membranes in Table 5 of Example 5 in Applicants' specification clearly crosses over and exceeds the upper limit of the pore size (0.1 μm) of ultrafiltration membranes, that pore sizes greater than 0.1 μm are clearly larger than the size of the RNA molecules isolated, and, therefore, that the data in Table 5 show that nucleic acid molecules are clearly being preferentially **bound** to the membrane and not simply retained by having a size larger than the pores of the membrane. Otherwise, in the absence of actually being bound to the membranes, the RNA molecules would not have been isolated but would have easily passed through the membranes with pore sizes of 0.2 μm, 0.45 μm, 0.65 μm, 1.2 μm, 5 μm, 10 μm, and 20 μm. Additionally, it was quite surprising that even after the elution the nucleic acid does not pass through the membrane. It is assumed that this unexpected effect results from the surface tension induced by the pores in combination with the liquid elution medium. This specific effect occurring during the isolation procedure could not have been foreseen by a person of ordinary skill in the art referring only to the cited ultrafiltration art. In fact, as shown in Table 5, noticeably high yields of purified RNA were obtained using membranes that had such pore sizes that range from two times (0.2 μm) to 200 times (20 μm) larger than the upper pore size limit of 0.100 μm used in standard ultrafiltration methods.

Put another way, the person of ordinary skill in this art would appreciate the difference between size exclusion methods of separation and a method of separation that relies on a binding reaction to a matrix with subsequent release. The Ogawa and now Ausich references relate to the former category of method; the present invention introduces a discovery in the latter category. And the teachings relating to size exclusion provide no insight or inspiration to the person of ordinary skill in the art applicable to

methods dependent on binding and release (and independent of matrix pore size, as demonstrated by Applicants' examples).

The above review of the various facts and explanations already present in this record clearly shows that there was no teaching, suggestion, or motivation -- let alone any reasonable expectation of success -- that membranes used to collect lycopene crystals according to Ausich could be successfully employed in the ultrafiltration of Ogawa to isolate nucleic acids. Only Applicants' disclosure provides the art with a process in which it is possible to employ non-siliceous membranes with pore sizes larger than would be useful for size exclusion separation of the target to isolate nucleic acids, **wherein the nucleic acids are bound to one side of the membrane in the presence of an immobilization buffer** as expressly recited in the claims and taught in the instant application.

Applicants note that the claimed invention cannot be obvious by merely combining elements and then assuming the combination will achieve the desired result. As the Federal Circuit in *In re Kotzab* noted:

> "A critical step in analyzing the patentability of claims pursuant to section 103(a) is **casting the mind back to the time of invention**, to consider the thinking of one of ordinary skill in the art, guided only by the prior art references and the then-accepted wisdom in the field. See *Dembiczak*, 175 F.3d at 999, 50 USPQ2d at 1617. Close adherence to this methodology is especially important in cases where the very ease with which the invention can be understood may prompt one 'to fall victim to the insidious effect of a hindsight syndrome wherein that which only the invention taught is used against its teacher.' *Id.* (quoting *W.L. Gore & Assocs. Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 313 (Fed.Cir.1983)).
>
> "Most if not all inventions arise from a combination of old elements. *See In re Rouffett*, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1457 (Fed.Cir.1998). Thus, every element of a claimed invention may often be found in the prior art. See *Id.* However, identification in the prior art of each individual part claimed is insufficient to defeat patentability of the whole claimed invention. See *Id.* Rather, **to establish obviousness based on a combination of the elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant**. *See In re Dance*, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed.Cir.1998); *In re Gordon*, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed.Cir.1984). . . .
>
> "The motivation, suggestion or teaching may come explicitly from statements in the prior art, the knowledge of one of ordinary skill in the art, or, in some cases the nature of the problem to be solved. *See Dembiczak*, 175 F.3d at 999, 50 USPQ2d at 1617. In addition, the teaching, motivation or suggestion may be implicit from the prior art as a whole, rather than expressly stated in the references. *See WMS Gaming,*

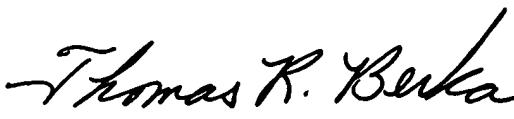
> *Inc. v. International Game Tech.*, 184 F.3d 1339, 1355, 51 USPQ2d 1385, 1397 (Fed. Cir. 1999).... Whether the Board relies on an express or an implicit showing, it must provide particular findings related thereto. *See Dembiczak*, 175 F.3d at 999, 50 USPQ2d at 1617." (*In re Kotzab*, 217 F.3d 1365, 1369-70, 55 USPQ2d 1313, 1316-17 (Fed. Cir. 2000) (emphasis added)).

As noted above, the motivation to combine may derive from many sources, however, the range of possible sources that may serve as evidence for a motivation to combine references "does not diminish the requirement for **actual evidence**. That is, the showing [of a motivation to combine] must be clear and particular." *In re Dembiczak*, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617, 1999 WL 246572 (Fed. Cir. 1999) (emphasis added). Furthermore, "[b]road conclusory statements standing alone are not 'evidence.'" *Id.*

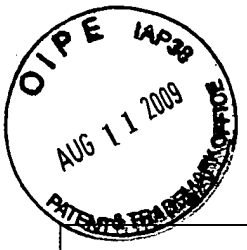
Conclusion

Nowhere has the Examiner presented the required evidence of a motivation to combine the cited references to make Applicants' claimed invention *prima facie* obvious. In fact, as explained above, the combination of Ausich with the primary reference Ogawa, with or without the other secondary references, is actually contrary to the teaching of Ogawa with respect to employing ultrafiltration to isolate nucleic acids. Moreover, the Examiner has not shown how such a combination would provide a person of ordinary skill in the art with a suggestion of Applicants' invention or how to achieve it in the absence of Applicants' own disclosure. Accordingly, the combination of references does not render Applicants' claims obvious under 35 USC § 103, and Applicants respectfully request that the Examiner reconsider and withdraw the rejections to pass Claims 1, 3-5, 9-17, 22, 24-31, 33-40, 51, 53-55, 59-64, 69-74, 76-81, and 83-100 to allowance.

Respectfully submitted,



Thomas R. Berka, Ph.D., Reg. No. 39,606
Leon R. Yankwich, Reg. No. 30,237
Attorneys for Applicants
Yankwich & Associates, P.C.
201 Broadway
Cambridge, Massachusetts 02139
telephone: 617-374-3700
facsimile: 617-374-0055



CERTIFICATE OF "EXPRESS MAIL" TRANSMISSION

The undersigned hereby certifies that this correspondence and accompanying documents are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR § 1.10, postage prepaid in an envelope addressed to the Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date indicated below.

Express Mailing No. **EV 509623077 US**

August 11, 2009
Date

M. F. Chinappi
Margaret F. Chinappi